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14. ABSTRACT
It is commonly held belief that prostate tumor invasion is triggered by the overproduction of proteolytic enzymes mainly by tumor cells, which cause degradation of the basement membrane. This theory is consistent with data from cell cultures and animal models, but results from recent worldwide clinical trials with enzyme inhibitors have been very disappointing, casting doubt on the validity of the enzyme theory. Based on our own studies, we have proposed that prostate tumor invasion is triggered by localized degeneration of aged or injured basal cells and the resultant auto-immunoreactions, which selectively favor aberrant proliferation and subsequent invasion of tumor stem or progenitor cells overlying focal basal cell layer disruptions. Our hypothesis differs from the traditional proteolytic enzyme theory in multiple aspects, including the stage of invasion, the precursor of invasive lesions, the roles of stromal and immunoreactive cells, and the potential approaches for prevention of invasion. Our hypothesis has been published in multiple peer-reviewed journals.

15. SUBJECT TERMS
Breast tumor invasion; Breast tumor progression; Myoepithelial cells; Early detection; Precursor of invasive breast cancer; Estrogen receptor expression; inflammatory cell infiltration.

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Introduction

The prostate luminal cells, which are the histological origin of a vast majority of prostate malignancies, are physically separated from the stroma by basal cells and the basement membrane (BM). Basal cells are joined by intercellular junctions and adhesion molecules, forming a continuous sheet that encircles ducts and acini (Fig 1) (1-2). The BM is composed of type IV collagen, laminins, and other molecules, forming a continuous lining surrounding and attaching to the basal cell layer (3-4). The epithelium is devoid of blood vessels and lymphatic ducts, and is therefore totally dependent upon the stroma for its normal functions and even survival. Due to this structural relationship, the disruption of both the basal cell layer and BM is pre-requisite for prostate tumor invasion or metastasis.

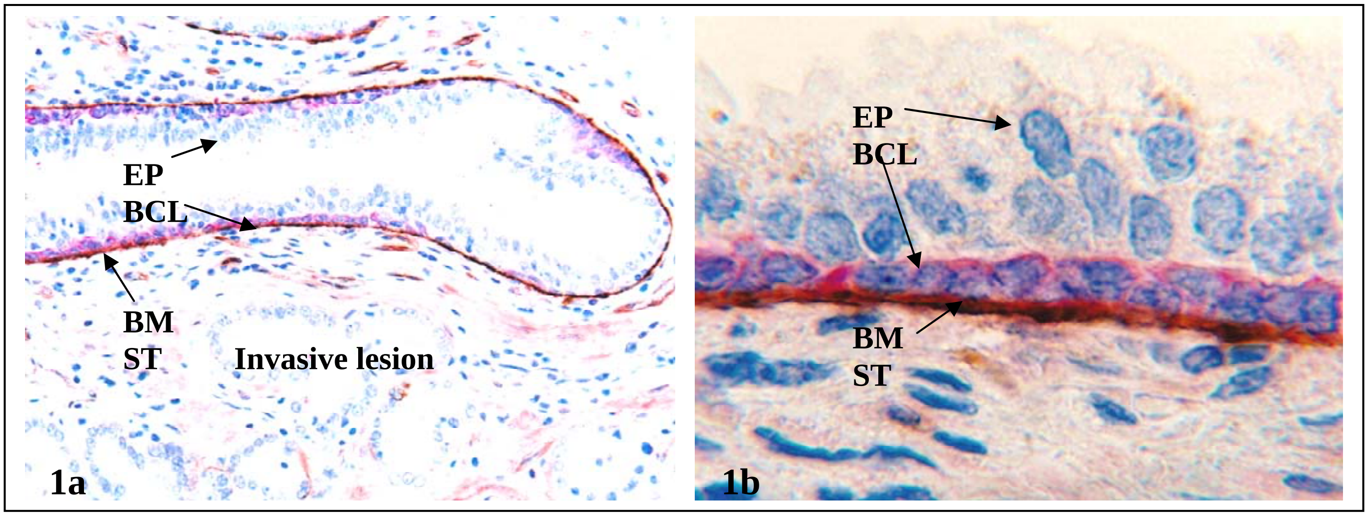


Fig 1. The structural relationship among the epithelium (EP), basal cell layer (BCL), basement membrane (BM), and stroma (ST). Paraffin-embedded human prostate tissue sections were double immunostained for the BM (brown) and basal cells (red). 1a: 200X 1b: a higher (800X) magnification 1a.

The development of prostate cancer is believed to be a multistep process, progressing sequentially from normal, to hyperplasia, to prostatic intraepithelial neoplasia (PIN), and to invasive lesions (5-6). The progression from PIN to invasive cancer is traditionally believed to be triggered primarily, if not solely, by the overproduction of proteolytic enzymes by cancer or stromal cells, which results in the degradation of the BM (7-8). Results from recent worldwide clinical trials with a wide variety of proteolytic enzyme specific inhibitors, however, have been very disappointing, casting doubt on the validity of the proteolytic enzyme theory (9-10). Since over 90% of prostate cancer related deaths result from invasion-related illness and the incidence of PIN could be up to 16.5% to 25% in routine or ultrasound guided prostate biopsy (11-12), there is an urgent need to uncover the intrinsic mechanism of prostate tumor invasion, and to define the specific tumors or individuals at greater risk for invasive lesions. It has been well documented that early detection and interventions could significantly improve prognosis and reduce treatment-related costs (13).

B: Preliminary Studies

Promoted by the reports that: [1] basal cells are the source of several tumor suppressors, including p63 and maspin, [2] the absence of basal cell layer is the most distinct feature of invasive lesions, and [3] chronic inflammation promotes prostate cancer (14-18), our recent studies have attempted to identify the early alterations of basal cell layers and their potential impact on prostate tumor invasion. Using a double ummunostaining method with antibodies to cytokeratin (CK) 34ßE12 (a basal cell phenotypic marker), our initial study assessed the physical integrity of basal call layers in paraffin-embedded tumor (n=50) prostate tissues with co-existing pre-invasive and invasive components (19). Of 2,047 ducts and acini examined, 201 were found to contain focal disruptions (the absence of basal cells resulting in a gap larger than the combined size of at least 3 basal cells) in surrounding basal cell layers. The frequency of focal disruptions (FBCLD)

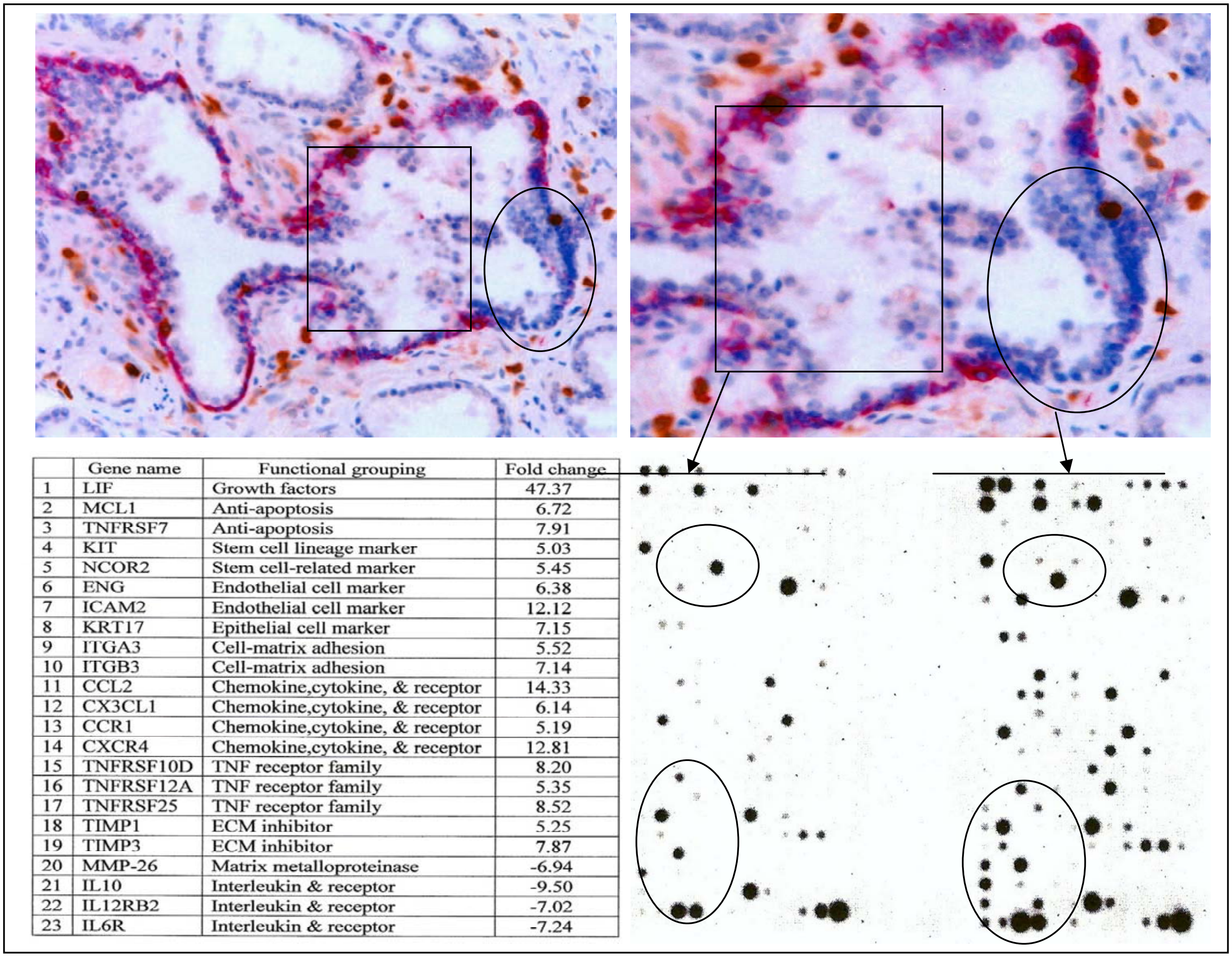
varied substantially among cases (Table 1).

Table 1. Frequencies of focal basal cell layer disruptions among different cases

Case number	No disruptions	1-10% disruptions	> 30% disruptions	p
50	22 (44%)	11 (22%)	17 (34%)	< 0.01

Compared to their non-disrupted counterparts, focally disrupted basal cell layers showed the following unique features: [1] significantly lower proliferation; [2] significantly lower p63 expression; [3] significantly higher apoptosis; [4] significantly higher leukocyte infiltration and stromal reactions.

Compared to their counterparts distant from focal disruptions or overlying the non-disrupted basal cell layers, epithelial cells overlying FBCLD showed the following unique features: [1] significantly higher proliferation; [2] significantly higher gene expression (Fig 2); [3] physical continuity with adjacent invasive lesions.



	Gene name	Functional grouping	Fold change
1	LIF	Growth factors	47.37
2	MCL1	Anti-apoptosis	6.72
3	TNFRSF7	Anti-apoptosis	7.91
4	KIT	Stem cell lineage marker	5.03
5	NCOR2	Stem cell-related marker	5.45
6	ENG	Endothelial cell marker	6.38
7	ICAM2	Endothelial cell marker	12.12
8	KRT17	Epithelial cell marker	7.15
9	ITGA3	Cell-matrix adhesion	5.52
10	ITGB3	Cell-matrix adhesion	7.14
11	CCL2	Chemokine,cytokine, & receptor	14.33
12	CX3CL1	Chemokine,cytokine, & receptor	6.14
13	CCR1	Chemokine,cytokine, & receptor	5.19
14	CXCR4	Chemokine,cytokine, & receptor	12.81
15	TNFRSF10D	TNF receptor family	8.20
16	TNFRSF12A	TNF receptor family	5.35
17	TNFRSF25	TNF receptor family	8.52
18	TIMP1	ECM inhibitor	5.25
19	TIMP3	ECM inhibitor	7.87
20	MMP-26	Matrix metalloproteinase	-6.94
21	IL10	Interleukin & receptor	-9.50
22	IL12RB2	Interleukin & receptor	-7.02
23	IL6R	Interleukin & receptor	-7.24

Fig 2. Comparison of gene expression between cells overlying focal BCLD and adjacent cells within the same duct. Cells were microdissected and sublected to RNA extraction, amplification, and gene expression profiling using our published protocols. Circles identify microdissected cells overlying focal BCLD & differentially expressed genes. Squares identify microdissected adjacent cells.

Among a total of 600 different genes assessed using the Pathway-focused oligo DNA micro-arrays, 23 genes were significantly and differently (at least 5-fold difference) expressed between cells overlying FBCLD and the adjacent cells within the same duct. Of these, genes-specific for extracellular matrix proteinases, interleukins

and their corresponding receptors were significantly lower in cells overlying FBCLD, which provides additional evidence that the proteolytic enzyme theory might not reflect the intrinsic mechanism of tumor invasion. In contrast, cells overlying FBCLD had significantly higher expression levels in several gene groups, including those for cell proliferation, anti-apoptosis, and stem cells (20; Fig 2). All these elevated genes have been shown to directly promote tumor progression and invasion.

Together, these findings suggest that focal basal cell layer disruptions could substantially impact the molecular profile and biological presentations of the overlying epithelial cells. Based on these and other findings, we have proposed that prostate tumor invasion is triggered by a localized degeneration of aged or injured basal cells and the resultant auto-immunoreactions. Our hypothesized steps for prostate tumor invasion include the following: [1] due to inherited or environmental factors, some patients contained cell cycle control- and renewal-related defects in the basal cell population that cause elevated basal cell degenerations; [2] the degradation products of degenerated basal cells or diffusible molecules of the overlying epithelial cells attract leukocyte infiltration; [3] leukocytes discharge their digestive enzymes upon the direct physical contact, resulting in a focal disruption in the basal cell layer, which leads to several focal alterations:

a. A localized loss of tumor suppressors and paracrine inhibitory function, which confers tumor cell growth advantages to escape the programmed cell death (21-25).
b. A localized increasing of permeability for nutrients and growth factors, and altered oxygen level, which selectively favors the proliferation of progenitor or stem cells (26-28).
c. A localized increasing of leukocyte infiltration, which directly export growth factors to tumor cells through direct physical contact (29-33).
d. The direct tumor-stromal cell contact, which augments the expression of stromal MMP or represses the expression of E-cadherin and other epithelial cell specific markers, which facilitates epithelial-mesenchymal transition (34-36).
e. The direct exposure of the overlying epithelial cells to the stromal tissue fluid, which might dilute the adhesion molecules on the surface of the epithelial cells.

These alterations could individually or collectively lead to increasing proliferation and motility in overlying epithelial cells that lead to the stromal invasion of the cells overlying FBCLD.

Our hypothesis differs from the traditional theories in six main aspects: [1] the triggering factor for the initiation of tumor invasion, [2] the stage of tumor invasion, [3] the cellular origin of invasive lesions, [4] the significance of immunoreactive cells, [5] the significance of stromal cells, and [6] potential approaches for early detection, treatment, and prevention of tumor invasion. Our hypothesis has been published in ***Medical Hypotheses 70: 387-408, 2008***. Our hypothesis represents a novel in vivo model as to the cellular mechanism leading to prostate tumor invasion. If confirmed, it could have an immediate impact on patient care through improved pathologic evaluation of prostate tumor biopsies. More broadly, the results of our study may lead to the development of more effective and specific approaches for prostate cancer detection, treatment, and prevention.

Key research accomplishments

1. All the laboratory procedures for all Tasks listed had been completed.
2. A total of 14 manuscripts and abstracts have been published, accepted, or submitted.
3. A total of 23 significantly and differentially (at least 5-fold) expressed genes have been identified between cells overlying focally disrupted basal cell layers and adjacent cells.
4. A novel hypothesis of prostate tumor progression and invasion has been published (Medical Hypotheses. 70: 387-408, 2008.

Reportable outcomes

A total of 14 manuscripts and abstracts have been published, accepted, or submitted, and two additional manuscripts are in preparation (please see below)

a. Manuscripts:

1. Man YG, Zhao CQ, Wang J, XL Chen. A subset of prostate basal cells lacks corresponding phenotypic markers. Pathology-Research & Practice, 202 (9): 651-662, 2006.
2. Man YG, Gardner WA. Focal basal cell degeneration and the resultant auto-immunoreactions: a potential mechanism for prostate tumor progression and invasion 70: 387-408, 2008.
3. Man YG, Sang QXA, Zhao CQ, Mannion C, Gardner WA. Focal basal cell degeneration induced lymphocyte infiltration is a potential trigger factor for prostate tumor invasion: implications for treatment and prevention. Cancer Detect Prev, in press
4. Man YG, Gardner WA. Bad seeds produce bad crops: a single-stage process of prostate carcinogenesis. Submitted.
5. Schwartz AM, Man YG, Rezaei MK, Simmens S, Berg PE. BP1. A homeoprotein, is significantly expressed in prostate adenocarcinomaand is concordant with Prostatic Intraepithelial Neoplasia (PIN). Submitted.
6. Man YG, Mason J, Prabhakar S, Wang B, Zeng X. Unique gene expression in cells overlying focally disrupted prostate basal cell layers. Manuscript in preparation.
7. Man YG, Zhang XC. Differential gene expression in focally disrupted and non-disrupted prostate basal cell layers. Manuscript in preparation.

b. Abstracts:

1. Man YG, Chen XL, Garcia FU, Gardner WA. Reduced p63 expression and elevated apoptosis in focally disrupted prostate basal cell layers: Implications for tumor invasion. Accepted for platform presentation at the 2006 USCAP Meeting. Lab Investigation 86:292A; 1358, 2006.
2. Man YG, Egland KA, Onda M, Nagata S, Pastan I. Expression of BPSR correlates with breast and prostate tumor progression and invasion. Proc Am Assoc Cancer Res 47: 2896, 2006
3. Man YG, Liu XF, Mason J, Prabhakar S, Wang B, Zeng X, Stamatakos, Gardner WA. Prostate tumor cells near and distant from focally disrupted basal cell layers have different gene expression profiles. 2006 American Society for Cell Biology Annual Meeting , 143:1843, 2006.
4. Nelson A, Tuteja A, Man YG. Inflammatory cells and membrane disruption in prostate carcinoma: Do they have a role in tumor invasion. Lab Investigation 87 (Supplement l): 166A, 2007.
5. Schwartz AM, Man YG, Rezaei MK, Berg PE. BP1, a homeoprotein, is significantly expressed in prostate adenocarcinoma and concordant with prostatic intraepithelial neoplasia. (AACR Annual Meeting. April 14-18, 2007. Los Angeles Convention Center, Los Angeles, CA). Proc Am Assoc Cancer Res 48: 162, 2007.
6. Man YG. Focal degeneration of aged or injured basal cells and resultant auto-immunoreactions are trigger factors for prostate tumor invasion. Accepted for poster presentation at the Department of Defence Prostate Cancer Research Program Meeting. Septermber 5-8, 2007. Atlanta, GA.
7. Liu AJ, Man YG, Gardner WA. Prostatic ducts and acini with and without focal disruptions in the basal cell layer have a different gene expression profile: Implications for tumor progression and invasion. American Society for Cell Biology 2007 Annual Meeting, December 1-5, Washington DC.
8. Man YG, Liu AJ, Gardner WA. Normal appearing prostate acinar and duct clusters with malignant profiles. Accepted for presentation at the United States and Canada Academe of Pathology (USCAP) 2008 Annual Meeting.
9. Man YG, Liu AJ, Gardner WA. Elevated tenascin expression in stroma near focally disrupted prostate basal cell layers: implications for tumor progression and invasion. Accepted for

presentation at the United States and Canada Academe of Pathology (USCAP) 2008 Annual Meeting.

Conclusions

The results of our current study are in total agreement with our previous hypothesis, further suggests that prostate tumor invasion is triggered by a localized degeneration of aged or injured basal cells and the resultant auto-immunoreactions.

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invasion. Cancer Detect Prev 29:161-69, 2005.

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